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Pharmacognostical study of the roots of the plants *Ziziphus oenoplia Mill.*

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ABSTRACT:
Aim of present study is to evaluate *Ziziphus oenoplia Mill* by itsPharmacognostically with different parameters in order to give possible scientific validation.The roots are astringent, bitter, antihelmintic,digestive, and antiseptic. They are useful for treating hyperacidity, ascaris infection, abdominal pain,and healing of wounds.A thick root was studied.The TranverseSection of the root was circular in outline. Outer cuticular layer or cork was followed by thin walled epidermal cells. Phellogen part was bilayered, Xylem around the well developed xylem calls. Biseriate, reddish orange coloured Medullary rays observed. vassals occupied the entire central portion and were traversed regularly by rows of medullary rays whose cells were lignified. The powder microscopy of the root powder reveals the presence of cork- about six layers, thin walled, tubular, polygonal cells.

Keywords: *Ziziphus oenoplia Mill*,xylem, Tranverse section

INTRODUCTION

These are the studies which deal with morphology, microscopy and Physico-chemical constituents of the plant material which has the pharmaco therapeutic property.[17] It is a throny shrub growing to about 1.5m in height.the leaves are simple and alternate ,ovate and lanceolate , acute and oblique. the flowers are green and subsessile axillary cymes .the fruit is globes drup, black and shiny when ripe containing a single seed.[18]

Distribution:
Indian subcontinent china and southeast asia.

Macrocopical characters :

The roots are astringent, bitter, antihelmintic,digestive, and antiseptic. They are useful for treating hyper acidity, ascaris infection, abdominal pain, and healing of wounds. It is a
spreading, sometimes climbing, thorny shrub growing to 1.5 m in height. The leaves are simple, alternate, ovate-lanceolate, acute and oblique. The flowers are green, in sub sessile axillary cymes. The fruit is a globose drupe, black and shiny when ripe, containing a single seed.[19,20,21]

Odour	**:**	Characteristic
Taste	**:**	Bitter, Astringent
Shape	**:**	Irregular
Width	**:**	1cm
Surface	**:**	Rough
Texture	**:**	Hard texture

Material And Methods

Plant Material

Roots of *Ziziphus oenoplia Mill* was collected from the road sides of Manas forest area, Panbazar road, 8-10 km from jorhat district of Assam, India, in the month of September 2011 in a quantity sufficient for all the experiments in a single batch.

Macroscopic study

Macroscopic structures of the root of *Ziziphus oenoplia Mill* were observed through naked eye. Simple microscope of magnification 10 (x10) was also used for the perception of special structural features.

Macroscopic structures

A thick root was studied. It is 1cm in diameter. The surface is rough and deeply fissured; the fissures are irregular in shape. The roots of *Ziziphus oenoplia Mill* having Characteristicodour, Bland taste and hard texture, yellowish brown Fracture.

Figure :- Morphological Features of *Ziziphus oenoplia Mill* root

Microscopic study

Introduction

Microscophy is an important tool in the evaluation of crude drugs which is applicable at various levels such as the authetication of crude drugs ,study of powdereded drugs ,calcium oxalate ,starch grains , Pollen grains etc .Ash values,extractive values and foaming index are used for the study of physical properties .[15,16]

Arrangement of plant into groups and sub groups is commonly spoken as classification .Various system of classifying plants have gradually developed during past few centuries ,which have emerged as a discipline of botanical science known as taxonomy or systemic botany .The word taxonomy is derived from two greek words taxis meaning as arrangement and nomas meaning laws . Therefore the systemisation of our knowledge about plants in and orderly manner becomes subject matter of systematic botany .

The aim and objective of taxonomy is to discover the similarities and differences in the plants including there closed relationship with their descents from common ancestry .It is a scientific way of naming , describing and arranging the plant in an orderly manner.

Materials and method for Anatomical studies

The Plant Care was taken to select healthy plants and for normal organs. The required samples of different organs were cut and removed from the plant and fixed in FAA (Formalin – 5ml + Acetic acid – 5ml + 70% Ethyl alcohol – 90ml). After 24 hrs of fixing, the specimens were dehydrated with graded series of tertiary-Butyl alcohol as per the schedule given by Sass, 1940. Infiltration of the specimens was carried by gradual addition of paraffin wax (melting point 58-60 C) until TBA solution attained super saturation. The specimens were cast into paraffin blocks.

Preparation of sample for section

Dried roots of *Ziziphus oenoplia Mill* was placed in a test tube containing water and boiled over Bunsen flame for a few minutes to soften the hard root

Sectioning

Transverse section was taken by cutting with a razor blade at right angle to the longitudinal axis of the root material. A thin section of the plant material (root) was mounted in chloral hydrate solution and warmed gently for clearing of the section, stained with phloroglucinol -hydrochloric acid (1:1) and mounted in glycerin . Cover slip was put with care to avoid air bubbles. The section was observed under using simple microscope and photograph was taken.The shape, size, colour, odour, taste, fracture roots were determined. Dried roots Powder was used for the observation of powder characteristics. The drug powder was separately treated with phloroglucinol-hydrochloric acid (1:1) solution.

Photomicrograph

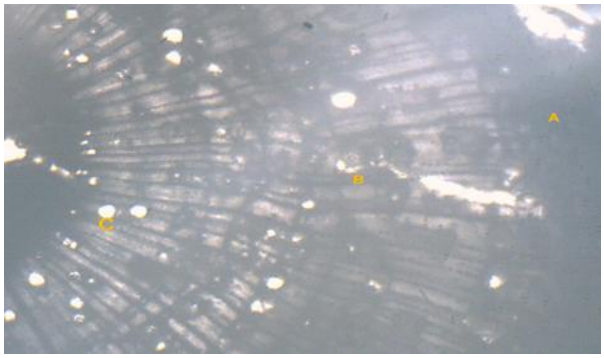
Microscopic description of tissues are supplemented with micrographs whenever necessary .photographs of different magnification of were taken with nikkon lab photo 2 microscopic unit . For normal observation bright field was used .For the study of crystals, starch grains and lignified cells, polarized light was employed. Since these structures have birefringent property, under polarized light they appear bright against dark background. Magnifications of the figures are indicated by the scale-bars. Descriptive terms of the anatomical features are as given in the standard Anatomy books .[12]

Anatomy of roots

Microscopic structures observed in sections

The TS of the root (Fig.3) was circular in outline. Outer cuticular layer or cork was followed by thin walled epidermal cells. Phellogen part was bilayered, immediately below the cork and cells tangentially elongated. Cortex portion of the root composed of 16- 18 layers of horizontally elongated parenchyma with small intercellular spaces. The parenchyma cells

contain starch grains which were both simple and compound. A continuous ring consists of phloem parenchyma. Phloem was seen in several thin patches .Xylem around the well developed xylem calls. Biseriate, reddish orange coloured Medullary rays observed. vessals occupied the entire central portion and were traversed regularly by rows of medullary rays those cells were lignified. Each ray cell was radially elongated and contains starch. The root section was shown absence of pith at the centre of the section.[11,12.]



T.S. of Young Root .(A- Cortex, B - xylem, C - Medullary rays,).

Powder microscopic characteristics

The powder microscopy of the root powder reveals the presence of cork- about six layers, thin walled, tubular, polygonal cells. Moss and liverwort cells, wood elements- xylem vessels and fibres inter lock with each other to form a spindle shaped structure, starch grains- simple as well as compound

Powder characteristics of roots

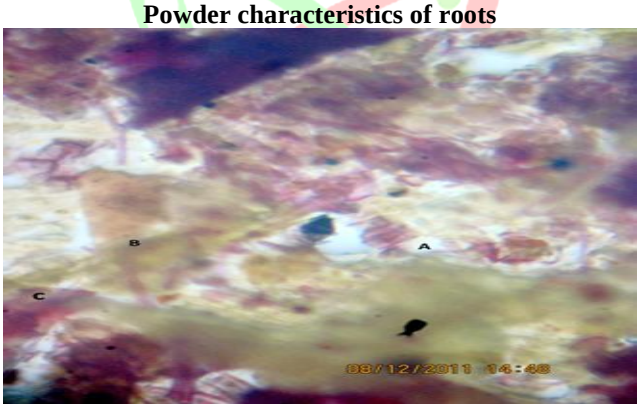


Figure :(A- Starch, B- Wood elements, C- Xylem)

Physical constant values

The physical constant evaluation of the drugs is an important parameter in detecting adulteration. Ash values are helpful in determining the quality and purity of crude drugs, especially in the powdered form. The object of ashing vegetable drugs is to remove all traces of organic matter which may otherwise interfere in an analytical determination. Extractive values are useful for (1) evaluation a crude drug, (2)give an idea about the nature of the chemical constituents present in a crude drug, and (3) useful for the estimation of specific constituents, soluble in that particular solvent used for extraction. The foaming ability of an aqueous decoction of plant materials and their extracts is measured in terms of a foaming index.

The ash values, extractive values and moisture content of root were determined. The results are depicted in Table 1**.** This study of Pharmacognostic including physico-chemical parameters is meant for proper identification of the plant, adulterant detection and also compilation of quality control standards of crude drugs. Though this plant have been a long history of traditional medicine for various human aliments, it is important to standardize it for use as a drug.

Physico-chemical constants

Ash values

Principle

The residue remaining after incineration of the crude drug is designed as ash. The residue obtained usually represents the inorganic salts naturally occurring in the drug and adhering to it .it varies with in definite limits according to the soils .it may also include inorganic matter added for purpose of adulteration .Hence an ash value determination furnishes the basis for juging the identity and of any drug and gives information relative to its adulteration /contamination with organic matter thus ash values are helpful in determining the quality and purity of the drug .The total ash values of crude drug reflects the care taken in its preparation .the acid insoluble ash is apart of the total ash that is insoluble in dilute hydrochloric acid .a higher limit of acid insoluble ash is imposed especially in cases where silica may be present when the calcium oxalate content of the drug is very high.Some analyst favour mixing of the sulphuric acid with the powered crude drug before ashing and this sulphated ash value is normally less fusible than ordinary ash .

Procedure given in Indian pharmacopoeia is used to determine the different ash values such as total ash acid insoluble ash water soluble ash value and sulphated ash value.[32,33]

(A) Determination of total ash value

Accurately weighed about 3 gms of air dried powered drug is taken in atared silica crucible and incerated by gradually increasing the temperature to make it dull red hot until free from carbon .Cooled and weighed repeated for contant value .then the percentage of total ash was calculated.

(B) Determination of acid insoluble ash value

The ash obtained as directed under total ash is boiled with 25 ml of 2N HCL for 5 mins .The insoluble matter was collected on an ash les than filter paper , washed with hot water dried with filter paper ignited and weighed .Then calculated the percentage of acid insoluble ash with reference to the air dried drug .

(C) Determination of water soluble ash value

The total ash obtained was boiled with 25ml of water for 5 minutes .

The insoluble matter is collected on an ash less filter paper , washed with hot water and ignited for 15 minutes at a tyempreature not exceeding 450^{0}C. The weighof insoluble matter was subtracted from the weigheh of total ash the difference in weigh represent the water insoluble ash .the percentage of water insoluble ash was calculated with with reference to the air dried drug .

(D) Determination of sulphated ash value

About 3 gm of accurately weighed air dried powdered drug was taken in a tared silica crucible , which was perviusly ignited and weighed. Then ignite gently at first until the drug was thoroughly charred . The crucible was cooled and residue was moisetened with 1ml of cocncentrated sulphuric acid ,heated gently until the white fumes were no longer evolved and ignited at 800 ^{0}C until all the black particles has disappear .the crucible was allowed to cool ,few drops of sulphuric acid was added and again heated . The ignition carried out as before allowed cooling and weighed to get constant weight (difference not more than 0.5gmbetween two consecutive readings) The percentage of sulphated ash was calculated with reference to air dried drugs . all the ash values were calculated and recorded .

Extractive values

Extract values of crude drugs are useful for the re-evaluation ,especially when the constituents of a drugs cannot be readily estimated by any other means futher this values indicate the nature of the constituents present in a crude drug. .The extractive values were determined by standard procedure .

Determination of alcohol soluble extractive value

5 gms of the air dried coarse powder of the roots of Ziziphus oenoplia Mill was macerated with 100ml of 90% ethanol in a closed flask for 24 hours shaking frequently during the first 6 hours and allow to stand for 18 hours .thereafter it was filter rapidly taking precaution against loss of solvent .Out of that filterate 25 ml of the filterate was evaporated to dryness in tared flat bottom shallow dish, dried at 105 ^{0}C and weighed. The percentage of ethanol soluble extractive value was calculated with reference to the air dried drug .and the results were recorded .

Determination of water soluble extractive value

Weigh accurately the 5 gms of coarsely powdered drug and macerate it with 100ml of chloroform water in a closed flask for 24 hours shaking frequently. During the first six hours and allowing to stand for 18 hours Thereafter it was filtered rapidly taking precaution against the loss of solvent .

Then 25ml of the filtrate was evaporated to dryness in tared flat bottomed shallow dish dried at 105^{0}C and weighed .The percentage of water soluble extractive was calculated with reference to the air dried drug and the results were recorded .

Loss on drying

Loss on drying is the loss in weight in %w/w determined by means of the procedure given below .It determines the amount of volatile matter of any kind (including water) that can be driven off under the condition specified (desiccator or hot air oven).if the sample is in the form of large crystals then reduce the size by quickly crushing it into a powder.

Procedure

About 1.5 gms of powered drug is weighed accurately in a tared porcelain dish which was previously dried at 105^0C in a hot air oven to constant weight and then weighed .from the difference in weight the percentage loss in drying with reference to the dried air substance was calculated and recorded.

Ash values

Ash value, acid insoluble ash, water soluble ash and loss on drying were determined as per standard Pharmacopoeal procedure.

TablePhysico- chemical values of root powder of *Ziziphus oenoplia Mill*

S.No.	Parameters	%w/w
1.	**Ash values**	
	a) Total Ash	10.00
	b) Acid insoluble Ash	2.86
	c) Water soluble ash	2.00
	d) Sulphated ash	2.1
2.	**Extractive Values**	
	a) Alcohol soluble extractive	7.5
	b) Water soluble extractive	6.8
3.	**Loss on drying**	16.8

Physical constant values like Total ash, acid insoluble ash, water soluble ash and loss on drying were reported. From the higher ash value suggested that the bark contains demonstrable quantity of inorganic salts and lower percentage acid insoluble ash suggest the presence of small quantity of calcium oxalate crystal. The smaller percentage loss on drying indicated that the bark contains very less amount of moisture.

Foaming index

Foaming index is mainly performed to determine the saponin content in an aqueous decoction of plant material .[13,14]

Determination of foaming index

Weigh accurately about 1 gms of coarse powedered drug and transfer to 500ml conical flask containing 100ml of boiling water maintained at a moderate boiling at 80- 90^0C for about 30 minutes .cooled and filtered in a volumetric flask added sufficient water in the filter to make up the volume to 100ml .cleaned 10 stopper test tube of uniform dimension were taken and marked from

1 to 10. Measured and transfer the successive portion of 1,2,3 ml upto 10ml and adjust the volume of liquid in each test tube with water to 10 ml stoppered the test tube and shaken them in a length wise motion for 15 seconds uniformly and allowed to stand for 15 mins and measure the height.

If the height of the foam in every tube is less than 1 cm the foaming index is less than 100(not significant) here the foam was more than 1 cm height after the dilution of the the plant material in the fourth tube the corresponding number of the test tube was the index sought .If the height of the foam in every test tube is more than 1 cm the foaming index is more than 1000 . In this case 10ml of the first decoction of the plant material is measured and transferred to 100ml volumetric flask and volume is made upto 100ml and followed the same procedure

Foaming index = 1000/a

Where a = volume of decoction used by preparing the dilution in the tube where exactly 1 cm or more foam was observed .the foaming index is calculated by using this formula and was tabulated.

Foaming index = 1000/5= 200

Thus the foaming index for the powdered roots of the *Ziziphus oenoplia Mill* was found to be 200.

FOAMING INDEX OF THE POWDERED ROOTS OF *ZIZIPHUS OENOPLIA MILL*

S.No	Test volumetric flask no.(10 ml)	Height of the foam (cm.)
1.	1	0.3
2.	2	0.6
3.	3	0.7
4.	4	0.9
5.	5	1.2
6.	6	1.4
7.	7	1.6
8.	8	1.6
9.	9	1.8
10.	10	1.9

Results And Discussion

The powdered roots of *Ziziphus oenoplia mill* belonging to family Rhamnaceae was selected for the project on the basis of ethano botanical information and availability .

Pharmacognostical Studies:

Macroscopic characters :

The roots are astringent, bitter, antihelmintic,digestive, and antiseptic. They are useful for treating hyperacidity, ascaris infection, abdominal pain,and healing of wounds.A thick root was studied. It is 1cm in diameter. The surface is rough and deeply fissured; the fissures are irregular in shape. The roots of *Ziziphus oenoplia mill* having Characteristic odour, Bland taste and hard texture, yellowish brown Fracture.

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Physiochemical parameters

Reported as total ash value acid insoluble ash value , water insoluble ash value sulphated ash value , water soluble extractive value and ethanol soluble extractive value and loss on drying . the above studies enable the identification of the plant material for future investigation and form an important aspect of drug studies .

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